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BRIEF REPORT

ACTA PÆDIATRICA NURTURING THE CHILD WILEY

The effects of Xenon gas inhalation on neuropathology in a placental-induced brain injury model in neonates: A pilot study

Improved obstetric and neonatal care have reduced the prevalence of severe hypoxic-ischaemic-encephalopathy (HIE). However, 1-3/1000 newborns in the developed world[1] suffer death or neurodevelopmental disability from HIE. The normal development of the brain during gestation can also be altered by placental reprogramming under oxidative stress. Under these conditions, the placenta releases DNA-damaging molecules, bone morphogenic proteins, microRNAs and glutamate.[2] At present, one is unable to diagnose or treat these factors.

We have previously applied Xenon, a rare noble gas used in anaesthesia, at 50% using a closed-circuit system with and without hypothermia in the newborn piglet and rodent models of HIE.[3,4] Unlike other inhalational anaesthetics, Xenon did not induce neuroapoptosis in the immature brain[5] and improved cardiovascular control after hypoxia-ischaemia (HI). A clinical feasibility and ongoing randomised phase-two study are testing the effects of breathing Xenon$^{50\%}$ in term infants undergoing therapeutic hypothermia (TH) against those undergoing TH alone. Experimentally, inhaling Xenon$^{50\%}$ improves motor function and cognition after long-term survival in rats post-injury.[4] In rat models of HI brain injury, Xenon is neuroprotective by upregulating neurotrophic factors and anti-apoptotic proteins,[6] by inducing hypoxia-inducible factor (HIF-1α) pathways allowing for pre-conditioning,[7] by suppressing the astroglial response to injury and limiting glutamate release to counter excitotoxicity thereby improving neuronal survival.[4]

Xenon's neuroprotective properties may be extended to treat brain injury arising from placental reprogramming under oxidative stress. To test this hypothesis, we used a rat injection model whereby media obtained directly from human placentae under oxidative stress were injected into postnatal day 4(P4) rat brain (human gestational age 29-31 weeks equivalent). In brief, media were collected from human first trimester placentae cultured under 21% O_2 (CM + 21%) and 2%-8% O_2 (CM + 2%-8%). An additional group was injected with saline (Sal) and constituted the sham condition. The pups were then allowed to survive into the juvenile age, brains were culled, and neuropathology was examined (see Supplementary files for more information).

We have tested (a) the effects of hypoxic injury modelled by the injection of hypoxia-derived conditioned media from the placenta into P4 rat pup brains and (b) whether breathing 50% Xenon for 4 hours after this injury could reduce neuropathology in those pups surviving into juvenile age.

We report here that the injection of hypoxia-derived conditioned media to healthy pups causes a modest loss of parvalbumin neurons in the thalamic reticular nucleus (TRN), the hippocampus and the cortex at P30 of survival (Figure 1). The glial response to neuronal loss was assessed by GFAP immunofluorescence and showed a marked increase in astrocytes in addition to an activated morphology. The injury also affected dendrite lengths, a proxy measure for degree of arborisation/connectivity, and this was consistent with previous in vitro findings.[8] There were no changes in overall neuronal counts, but dopaminergic neurons process lengths decreased in some areas. Importantly, Xenon treatment conferred some resistance to the increase in glial numbers ($P < 0.05$) in the cortex and hippocampus (Figure 1). Most strikingly, we observed Xenon treatment to be protective against the loss of parvalbumin cells in the TRN caused by the injury ($P < 0.05$). Interestingly, Xenon treatment did not protect dendritic arborisations/complexity but did greatly increase the lengths of dopaminergic (tyrosine hydroxylase) processes and overall neuronal numbers post-injury.

These promising results albeit limited in scope support that Xenon treatment after mild injury due to maternal hypoxia does offer some protection. Our findings are consistent with previously reported effects of Xenon in toning down gliosis in neonatal rat cortex, hippocampus and thalamus in a classical HI rodent model.[9,10] Most neurons including parvalbumin neurons are sensitive to hypoxia and are lost in the 2%-8% condition in most areas. Xenon acts as an anti-apoptotic agent, and as neurogenesis is still very active in the early developing postnatal rodent brain, Xenon may be promoting a compensatory neuroblast differentiation response in vulnerable areas explaining the higher number of densities we observed compared with 21%. This is speculative, and we do not know the underlying mechanisms. We also cannot speculate on the behavioural significance of an overall increased number of neurons without further study. Xenon is thought to provide partial protection of dopaminergic cells by acting as a trophic factor in conditions of excitotoxicity and by suppressing the astroglial response.[11] This improves cell survival but may also result in outgrowth around the area of damage. Fibre outgrowth is linked with altered connectivity in the brain and, therefore, may not necessarily beneficial. Behavioural work is needed to test how motor skills have been affected with/ without Xenon after

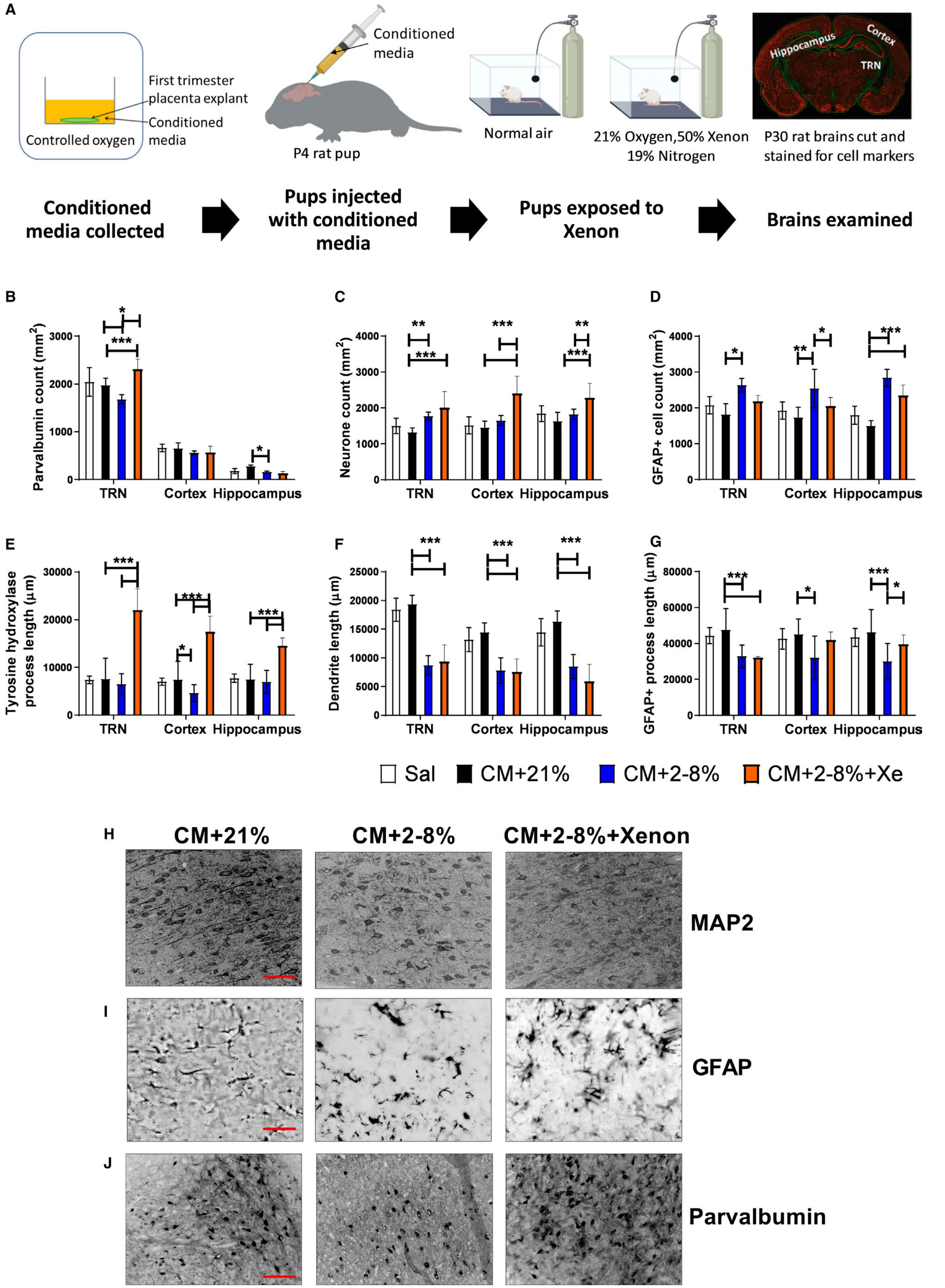
A
Controlled oxygen
First trimester placenta explant
Conditioned media
Conditioned media
P4 rat pup
Normal air
21% Oxygen,50% Xenon 19% Nitrogen
Hippocampus
Cortex
TRN
P30 rat brains cut and stained for cell markers
Conditioned media collected
Pups injected with conditioned media
Pups exposed to Xenon
Brains examined
B
Parvalbumin count (mm²)
C
Neurone count (mm²)
D
GFAP+ cell count (mm²)
E
Tyrosine hydroxylase process length (μm)
F
Dendrite length (μm)
G
GFAP+ process length (μm)
TRN
Cortex
Hippocampus
Sal
CM+21%
CM+2-8%
CM+2-8%+Xe
H
CM+21%
CM+2-8%
CM+2-8%+Xenon
MAP2
I
GFAP
J
Parvalbumin

FIGURE 1 Methodology and effect of placental hypoxic secretions with/without Xenon treatment on neuropathology. (A) Diagram of experimental set-up (see Supplementary files). Placental explants were incubated for 24 hours at either 21% or 2% oxygen conditions in trophoblast media and maintained in neurotrophic medium at 21% or at 2%-8%. Media were collected according to two conditions: CM + 21% (control) and CM + 2%-8% (hypoxia/reoxygenation). Media were injected into the brain of P4 rats (n=3/condition) and an n=3 was injected with saline as the sham condition). 3 pups from the CM + 2%-8% condition were subsequently placed in a closed-loop system at Xenon$^{50\%}$ for 4 hours. Pups were kept in normal conditions until P30. The P30 brains were collected, fixed and assessed for neuropathology. All identifiers were hidden for assessment and de-blinded for statistical analyses. (B-G) Results from cell density quantifications in specific anatomical areas such as the thalamic reticular nucleus, cortex and hippocampus at P30: (B) Parvalbumin neuron densities; (C) MAP2 neuron densities; (D) GFAP astrocyte densities. We also report process lengths for dopaminergic cells (E), MAP2 neurons (F) and astrocytes (G) process lengths in the thalamic reticular nucleus, hippocampus and cortex. Testing was done using a two-way ANOVA with Tukey's post hoc test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; n=3/group; data shown as means ± SD). (H-J) Representative photomicrographs of MAP2 (H) and GFAP (I) cells in the cortex, parvalbumin cells (J) in the reticular nucleus of pups injected with conditioned media from placental explants cultured at 21% and 2%-8% oxygen with/without Xenon treatment. Images were converted to grey scale and inverted for clarity using ImagePro Premier. Scale bar = 40 μm. *CM = conditioned media*

injury. Although we did not perform cognitive tests on the rodents, no significant neuropathology in the tissues was detected. Previously, when Xenon was administered shortly after a carotid ligation + hypoxic insult on P7 rat pups (near-term equivalent), which survived into adulthood, both neuropathology and behaviural testing were improved by Xenon$^{50\%}$.[4] In this 10-week survival to adulthood model, we defined that the time-window for neuroprotection with Xenon was 5 hours, which is the basis for our clinical trial of administering Xenon within 5 hours after birth since delayed Xenon significantly improved outcome. Another clinical trial delivered Xenon$^{30\%}$ starting at 10 hours of age and short-term outcome (using Magnetic Resonance Spectroscopy) after 10 days did not show any difference between TH and TH + Xenon.[12] It is likely that any effect of Xenon on cognition needs long-term follow-up. We are currently undertaking full IQ testing at 3-5 years in the CoolXenon trial (ending 010321).

Several clinical conditions present with hypoxic insults to the placenta including pre-eclampsia, maternal gestational diabetes[13] and stress.[14] Placental reprogramming can alter fetal neurodevelopment due to maternal hypoxia and Xenon inhalation may yet offer a promising therapeutic strategy.

KEYWORDS

brain injury, cooling, hypoxic-ischaemic encephalopathy, neurodevelopment, placental reprogramming, Xenon

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.